A GROWTH-DELAYING EFFECT OF ULTRAVIOLET RADIATION ON BACTERIAL VIRUSES

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Ultraviolet and ionizing radiations are known to inactivate bacterial viruses (bacteriophages).[1, 2] The inactivated virus no longer multiplies on sensitive bacteria or causes lysis. In the case of ultraviolet radiation, it has been found in some instances that the inactivated virus still retains the ability to be adsorbed by sensitive bacteria, and to interfere both with their viability and with their ability to support growth of active virus.[3] This residual property of irradiated virus is itself destroyed by higher doses of radiation.

Radiation is supposed to produce mutations in viruses.[4] In the course of attempts to increase by irradiation the rate of occurrence of mutations affecting the host range of bacterial viruses,[5] we encountered a new type of physiological, non-hereditary effect of ultraviolet radiation, namely, a growth-delaying effect, which will be described in the present communication.

Bacterial viruses grow by infecting living bacterial cells of sensitive strains and multiplying inside the cells. After a fairly constant interval of time, the cells are lysed and liberate a large number of virus particles.[6] We can describe the process in terms of a series of parameters: rate of virus adsorption by bacteria; "constant period," i.e., minimum interval between infection and lysis; "rise period," i.e., spread in the values of this interval for individual cells; "burst size," i.e., average yield of virus particles per lysed cell. These parameters are easily reproducible under given conditions for each virus-host system. Methods for their determination have been described in detail.[7]

The radiation effect with which we are dealing was discovered when we studied by these methods the growth of the fraction of irradiated virus that had survived ultraviolet irradiation. Filtrates of various bacterial viruses, all active on a common host, *Escherichia coli B*, were irradiated in thin layers (average about 0.02 mm.) with ultraviolet light containing about 80% of 2537 Å. radiation. The virus surviving various times of irradiation was then titrated and, within a few minutes, growth experiments of this surviving virus were performed. Our conditions of irradiation, although not such as to eliminate all screening effects by components of the medium, were, however, accurate enough to give reproducible amounts of inactivation.

The three viruses studied, α, γ and $T7$, are known to be unrelated on the

basis of serological and cross-resistance tests. Viruses α and $T7$ consist of small particles, virus γ of large particles, as shown by electron micrography and x-ray sensitivity tests.[8, 9] Upon ultraviolet irradiation, virus γ proved the most sensitive, virus $T7$ more resistant, virus α very resistant. For the same percentage of inactivation, the relative doses for the three viruses, measured in time of exposure, were in the approximate ratios of 1:4:10.

In the growth experiments using the virus surviving irradiation, adsorption by sensitive bacteria proved normal. When the constant periods were measured, however, they were found to be longer than for normal virus. The rise period was also longer than normal, whereas the burst size was normal. The values for the constant period are shown in table 1.

TABLE 1

	TIME OF IRRADIATION, MIN.	RESIDUAL ACTIVITY, %	CONSTANT PERIOD OF GROWTH ON *Escherichia coli B* (IN NUTRIENT BROTH AT 37°C.), MIN.
Virus α	0	100	13
	1	20	18
	3	2	25
	6	0.3	31
Virus γ	0	100	21
	0.5	1	24
Virus $T7$	0	100	13
	1	7	23
	5	0.2	28

Rise period and burst size were only determined in a few cases, because it is difficult to run complete growth experiments with low initial virus titers. We had to avoid the use of concentrated virus suspensions, in order to exclude the possibility of interference effects from the inactive virus particles: all experiments were done with bacteria in excess of the total amount of virus (active + inactive). The increase in rise period, however, was clearly shown in all cases by changes in the slope of the growth curve of virus.

As a whole, the effect appears to consist of a delay in virus liberation, without change in virus yield per bacterium. Not only is the minimum time for lysis longer, but the intervals between infection and lysis for individual bacteria are also more variable, as shown by the increase of the rise period. Both constant period and rise period are progressively more affected, for each virus, by increasing doses of radiation.

Similar results were obtained if the irradiated virus was diluted and stored at 5°C. for one day between irradiation and the growth experiment. The effect proved, however, strictly non-hereditary: the offspring of the irradiated virus, when tested in its turn, grew on sensitive bacteria in the same way as normal virus.

It is seen in table 1 that the growth-delaying effect of radiation, while increasing with the dose of radiation, does not parallel the inactivation effect on the various viruses: for comparable amounts of inactivation, growth delay is pronounced for the slowly inactivated viruses α and $T7$, barely evident for the very sensitive virus γ. It appears that the growth-delaying effect is more closely related, for various viruses, to the total time of irradiation, that is, to the number of quanta absorbed per unit of volume. Since the delay effect must be brought about by the action of quanta which have not acted lethally, and since it is a typically progressive effect, increasing in intensity with increasing doses of radiation, one is led to conclude that it results from a cumulative effect of ultraviolet quanta on the body of the virus particles.

Upon further irradiation, these particles would then become inactivated. Inactivation is likely to be caused, not by the cumulative effect of a larger number of quanta, but by a single "effective hit," as is true in the case of ionizing radiation, and as seems suggested by Gates's results with ultraviolet light.[1] A discussion of the mechanism of inactivation is, however, beyond the scope of the present paper.

The discovery of a physiological effect of non-lethal ultraviolet quanta on the particles of bacterial viruses raised an interesting question as to the occurrence of a similar effect for x-rays. It has been conclusively proved[8, 9] that in the case of x-rays nearly each "hit," that is, each elementary act of absorption of radiation in a particle of a bacterial virus, is effective in producing inactivation. A comparison of the "sensitive volumes" with the actual volumes of the particles shows that the ratio of hits to effective hits is not larger than 3:1 or 4:1, and is very likely close to 1:1. Under such conditions, we should hardly expect any non-lethal effect of radiation energy absorbed by the particles, comparable to the effect described above for ultraviolet light.

This expectation was proved to be correct in a series of experiments in which we exposed viruses α and γ to doses of x-rays (200 kv.) sufficient to reduce their titers to 0.1–5 per cent, and then studied the growth of the surviving virus. In all cases the growth was completely normal: constant period, rise period and burst size were like those of non-irradiated controls. It is clear that no delaying effect on growth is produced by x-rays, as expected in view of the high yield of inactivation by each act of absorption.

The effect of ultraviolet light described in this paper is an example of a non-lethal, non-genetic alteration of virus particles produced by radiation. The change involved affects the time interval between adsorption of a virus particle by a sensitive host and liberation of new virus. It is impossible to decide at present which of the various steps involved in the process leading to virus liberation is affected, whether it is the speed of penetration into the bacterial cell, or the rate of multiplication inside the

cell, or the reactions responsible for cell lysis. A delay in any one or more of these steps would account for the result. It is worth recalling that non-lethal, growth-delaying effects of ultraviolet and ionizing radiation are of familiar occurrence in the case of bacteria, yeasts, protozoa and cells of higher organisms. The growth-delaying effect here described may well be the expression of the same phenomenon in bacterial viruses.

The actual nature of the structural change causing growth delay is also open to speculation. Since bacterial viruses seem to be composed of nucleoproteins [10, 11] a study of the effectiveness of ultraviolet light of different wave-lengths, differently absorbed by specific components of nucleoproteins, might throw some light on this question.

In conclusion, it is seen that virus particles, in spite of their small size and probably simple composition, can undergo, under the influence of ultraviolet radiation, structural changes of various kinds. Of these, some affect the reproducing capacity of the virus; others even destroy the ability of the virus to be adsorbed by the host cells and to interfere with the growth of other virus particles; other changes may hereditarily affect some of the properties of the virus particles; finally, changes of the kind here described alter only the rate of some reactions involved in virus reproduction, in a strictly individual, non-hereditary manner.

The growth-delaying effect of radiation, detected for bacterial viruses, may well occur with other groups of viruses. In most of them, however, such an effect would be difficult to detect, since only for bacterial viruses can we study an isolated step of growth of a given group of virus particles. Radiation effects of this type may be of some relevance in the study of virus attenuation, partial neutralization, vaccine production and similar problems.

Summary.—Ultraviolet radiation, besides inactivating bacterial viruses, produces a delay in the growth of the surviving virus particles on a sensitive host; the delay increases with increasing doses of radiation. This effect is non-hereditary, and differently pronounced for different viruses. It appears to be due to the cumulative effect of the quanta absorbed by the virus particles before their inactivation. No similar effect was found on virus particles which had survived x-ray irradiation. This is explained by the fact that, for ionizing radiation, nearly each act of absorption by a virus particle is effective in producing inactivation: the surviving particles are likely not to have absorbed any radiation at all.

* Part of the experiments described in this paper were done in the summer of 1944 in the Department of Genetics of the Carnegie Institution of Washington, Cold Spring Harbor, New York.

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